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(54) Title: POLYPEPTIDES FROM NEISSERIA MENINGITIDIS

(57) Abstract: Various specific meningococcal proteins are disclosed. The invention provides related polypeptides, nucleic acids, antibodies and methods. These can all be usedin medicine for treating or preventing disease and/or infection caused by meningococcus, such as bacterial meningitis.

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POLYPEPTIDES FROM NEISSERIA MENINGITIDIS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of *Neisseria meningitidis.*

BACKGROUND ART

Neisseria meningitidis (meningococcus) is a non-motile Gram negative diplococcus that is pathogenic in humans. It colonises the pharynx and causes meningitis (and, occasionally, septicaemia in the absence of meningitis).

All pathogenic meningococci have a polysaccharide capsule. These polysaccharides form the bassis of available vaccines against meningococcus serogroups A, C, W135 and Y, but they are inappropriate for use against serogroup B. There has thus been a great deal of research into identifying alternative antigens for immunising against serogroup B. Such alternatives have included proteins, the lipopolysaccharide, and outer membrane vesicles.

References 1 to 7 disclose various polypeptides derived from the genome sequence of a serogroup B meningococcus, and they select specific sequences for use in vaccines. Genome sequence for a serogroup A strain is disclosed in reference 8.

It is an object of the invention to provide further polypeptides for use in the development of vaccines for preventing and/or treating meningococcal infections. In particular, it is an object to provide polypeptides for use in improved vaccines for preventing and/or treating meningococcal meningitis. The polypeptides may also be useful for diagnostic purposes, and as targets for antibiotics.

DISCLOSURE OF THE INVENTION

Polypeptides

The invention provides polypeptides comprising the meningococcal amino acid sequences disclosed in the examples. These amino acid sequences are the even SEQ ID NOs between 2 and 78. There are thus 39 amino acid sequences, and these are referred to as B269_*nn*, where *nn* is a number between 01 and 50 (there are eleven B269_*nn* numbers that have no sequence: 02, 03, 04, 05, 06, 07, 08, 09, 10, 12 & 40). Two preferred sequences are B269_32 and B269_37.

The invention also provides polypeptides comprising amino acid sequences that have sequence identity to the meningococcal amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*e.g.* 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more). These polypeptides include homologs, orthologs, allelic variants and functional mutants. Typically, 50% identity or more between two polypeptide sequences is considered to be an indication of functional equivalence. For any particular SEQ ID, the degree of sequence identity is preferably greater than both of the values in columns (B) and (A) of Table II herein, and is more preferably greater than all of the values in columns (C), (B) and (A) for that SEQ ID.

These polypeptide may, compared to the meningococcal sequences of the examples, include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) conservative amino acid replacements *i.e.* replacements of one amino acid with another which has a related side chain. Genetically-encoded amino acids are generally divided into four families: (1) acidic *i.e.* aspartate, glutamate; (2) basic *i.e.* lysine, arginine, histidine; (3) non-polar *i.e.* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar *i.e.* glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In general, substitution of single amino acids within these families does not have a major effect on the biological activity. The polypeptides may also include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) single amino acid deletions relative to the meningococcal sequences of the examples. The polypeptides may also include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) insertions (*e.g.* each of 1, 2, 3, 4 or 5 amino acids) relative to the meningococcal sequences of the examples.

The invention further provides polypeptides comprising fragments of the meningococcal amino acid sequences disclosed in the examples. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more).

The fragment may comprise at least one T-cell or, preferably, a B-cell epitope of the sequence. T- and B-cell epitopes can be identified empirically (*e.g.* using PEPSCAN [9,10] or similar methods), or they can be predicted (*e.g.* using the Jameson-Wolf antigenic index [11], matrix-based approaches [12], TEPITOPE [13], neural networks [14], OptiMer & EpiMer [15, 16], ADEPT [17], Tsites [18], hydrophilicity [19], antigenic index [20] or the methods disclosed in reference 21 *etc.*). Other preferred fragments are (a) the N-terminal signal peptides of the meningococcal polypeptides of the invention, (b) the meningococcal polypeptides, but without their N-terminal signal peptides, (c) the meningococcal polypeptides, but without their N-terminal amino acid residue.

Polypeptides of the invention can be prepared in many ways e.g. by chemical synthesis (in whole or in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (*e.g.* from recombinant expression), from the organism itself (*e.g.* after bacterial culture, or direct from patients), *etc.* A preferred method for production of peptides <40 amino acids long involves *in vitro* chemical synthesis [22,23]. Solid-phase peptide synthesis is particularly preferred, such as methods based on tBoc or Fmoc [24] chemistry. Enzymatic synthesis [25] may also be used in part or in full. As an alternative to chemical synthesis, biological synthesis may be used *e.g.* the polypeptides may be produced by translation. This may be carried out *in vitro* or *in vivo*. Biological methods are in general restricted to the production of polypeptides based on L-amino acids, but manipulation of translation machinery (*e.g.* of aminoacyl tRNA molecules) can be used to allow the introduction of D-amino acids (or of other non natural amino acids, such as iodotyrosine or methylphenylalanine, azidohomoalanine, etc.) [26]. Where D-amino acids are included, however, it

is preferred to use chemical synthesis. Polypeptides of the invention may have covalent modifications at the C-terminus and/or N-terminus.

Polypeptides of the invention can take various forms (*e.g.* native, fusions, glycosylated, non-glycosylated, lipidated, non-lipidated, phosphorylated, non-phosphorylated, myristoylated, non-myristoylated, monomeric, multimeric, particulate, denatured, *etc.*).

Polypeptides of the invention are preferably provided in purified or substantially purified form *i.e.* substantially free from other polypeptides (*e.g.* free from naturally-occurring polypeptides), particularly from other meningococcal or host cell polypeptides, and are generally at least about 50% pure (by weight), and usually at least about 90% pure *i.e.* less than about 50%, and more preferably less than about 10% (*e.g.* 5%) of a composition is made up of other expressed polypeptides. Polypeptides of the invention are preferably meningococcal polypeptides. Polypeptides of the invention preferably have the function indicated in Table I for the relevant sequence.

Polypeptides of the invention may be attached to a solid support. Polypeptides of the invention may comprise a detectable label (*e.g.* a radioactive or fluorescent label, or a biotin label).

The term "polypeptide" refers to amino acid polymers of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, *etc.*), as well as other modifications known in the art. Polypeptides can occur as single chains or associated chains. Polypeptides of the invention can be naturally or non-naturally glycosylated (*i.e.* the polypeptide has a glycosylation pattern that differs from the glycosylation pattern found in the corresponding naturally occurring polypeptide).

The invention provides polypeptides comprising a sequence -X-Y- or -Y-X-, wherein: -X- is an amino acid sequence as defined above and -Y- is not a sequence as defined above *i.e.* the invention provides fusion proteins. Where the N-terminus codon of a polypeptide-coding sequence is not ATG then that codon will be translated as the standard amino acid for that codon rather than as a Met, which occurs when the codon is a start codon.

The invention provides a process for producing polypeptides of the invention, comprising the step of culturing a host cell of to the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, wherein the polypeptide is synthesised in part or in whole using chemical means.

The invention provides a composition comprising two or more polypeptides of the invention.

The invention also provides a hybrid polypeptide represented by the formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein X is a polypeptide of the invention as defined above, L is an optional linker amino

acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and n is an integer greater than 1. The value of n is between 2 and x, and the value of x is typically 3, 4, 5, 6, 7, 8, 9 or 10. Preferably n is 2, 3 or 4; it is more preferably 2 or 3; most preferably, $n = 2$. For each n instances, -X- may be the same or different. For each n instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when n=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* Gly_n where $n = 2, 3, 4, 5, 6, 7, 8, 9, 10$ or more), and histidine tags (*i.e.* His_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. -A- and -B- are optional sequences which will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct polypeptide trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more). Other suitable N-terminal and C-terminal amino acid sequences will be apparent to those skilled in the art.

Various tests can be used to assess the *in vivo* immunogenicity of polypeptides of the invention. For example, polypeptides can be expressed recombinantly and used to screen patient sera by immunoblot. A positive reaction between the polypeptide and patient serum indicates that the patient has previously mounted an immune response to the protein in question *i.e.* the protein is an immunogen. This method can also be used to identify immunodominant proteins.

Antibodies

The invention provides antibodies that bind to polypeptides of the invention. These may be polyclonal or monoclonal and may be produced by any suitable means (*e.g.* by recombinant expression). To increase compatibility with the human immune system, the antibodies may be chimeric or humanised (*e.g.* refs. 27 & 28), or fully human antibodies may be used. The antibodies may include a detectable label (*e.g.* for diagnostic assays). Antibodies of the invention may be attached to a solid support. Antibodies of the invention are preferably neutralising antibodies.

Monoclonal antibodies are particularly useful in identification and purification of the individual polypeptides against which they are directed. Monoclonal antibodies of the invention may also be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA), *etc.*. In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme. The monoclonal antibodies produced by the above method may also be used for the molecular identification and characterization (epitope mapping) of polypeptides of the invention.

Antibodies of the invention are preferably provided in purified or substantially purified form. Typically, the antibody will be present in a composition that is substantially free of other polypeptides *e.g.* where less than 90% (by weight), usually less than 60% and more usually less than 50% of the composition is made up of other polypeptides.

Antibodies of the invention can be of any isotype (*e.g.* IgA, IgG, IgM *i.e.*
but will generally be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. Antibodies of the invention

Antibodies of the invention can take various forms, including whole antibodies, antibody fragments such as F(ab')2 and F(ab) fragments, Fv fragments (non-covalent heterodimers), single-chain antibodies such as single chain Fv molecules (scFv), minibodies, oligobodies, *etc.* The term "antibody" does not imply any particular origin, and includes antibodies obtained through non-conventional processes, such as phage display.

The invention provides a process for detecting polypeptides of the invention, comprising the steps of: (a) contacting an antibody of the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

The invention provides a process for detecting antibodies of the invention, comprising the steps of: (a) contacting a polypeptide of the invention with a biological sample (*e.g.* a blood or serum sample) under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Nucleic acids

The invention provides nucleic acid comprising the meningococcal nucleotide sequences disclosed in the examples. These nucleic acid sequences are the odd SEQ ID NOs between 1 and 77.

The invention also provides nucleic acid comprising nucleotide sequences having sequence identity to the meningococcal nucleotide sequences disclosed in the examples.

The invention also provides nucleic acid which can hybridize to the meningococcal nucleic acid disclosed in the examples. Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase stringency of a hybridization reaction of widely known and published in the art [*e.g.* page 7.52 of reference 29]. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C, 55°C and 68°C; buffer concentrations of 10 x SSC, 6 x SSC, 1 x SSC, 0.1 x SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or de-ionized water. Hybridization techniques and their optimization are well known in the art [*e.g.* see references 29-32, *etc.*].

In some embodiments, nucleic acid of the invention hybridizes to a target of the invention under low stringency conditions; in other embodiments it hybridizes under intermediate stringency conditions; in preferred embodiments, it hybridizes under high stringency conditions. An exemplary set of low stringency hybridization conditions is 50°C and 10 x SSC. An exemplary set of intermediate stringency hybridization conditions is 55°C and 1 x SSC. An exemplary set of high stringency hybridization conditions is 68°C and 0.1 x SSC.

Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least *n* consecutive nucleotides from the meningococcal sequences and, depending on the particular sequence, *n* is 10 or more (*e.g.* 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more).

The invention provides nucleic acid of formula 5'-X-Y-Z-3', wherein: -X- is a nucleotide sequence consisting of *x* nucleotides; -Z- is a nucleotide sequence consisting of *z* nucleotides; -Y- is a nucleotide sequence consisting of either (a) a fragment of one of the odd-numbered SEQ ID NOS: 1 to 77, or (b) the complement of (a); and said nucleic acid 5'-X-Y-Z-3' is neither (i) a fragment of one of the odd-numbered SEQ ID NOS: 1 to 77 nor (ii) the complement of (i). The -X- and/or -Z- moieties may comprise a promoter sequence (or its complement).

The invention also provides nucleic acid encoding the polypeptides and polypeptide fragments of the invention.

The invention includes nucleic acid comprising sequences complementary to the sequences disclosed in the sequence listing (*e.g.* for antisense or probing, or for use as primers), as well as the sequences in the orientation actually shown.

Nucleic acids of the invention can be used in hybridisation reactions (*e.g.* Northern or Southern blots, or in nucleic acid microarrays or 'gene chips') and amplification reactions (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA, *etc.*) and other nucleic acid techniques.

Nucleic acid according to the invention can take various forms (*e.g.* single-stranded, double-stranded, vectors, primers, probes, labelled *etc.*). Nucleic acids of the invention may be circular or branched, but will generally be linear. Unless otherwise specified or required, any embodiment of the invention that utilizes a nucleic acid may utilize both the double-stranded form and each of two complementary single-stranded forms which make up the double-stranded form. Primers and probes are generally single-stranded, as are antisense nucleic acids.

Nucleic acids of the invention are preferably provided in purified or substantially purified form *i.e.* substantially free from other nucleic acids (*e.g.* free from naturally-occurring nucleic acids), particularly from other *Haemophilus* or host cell nucleic acids, generally being at least about 50% pure (by weight), and usually at least about 90% pure. Nucleic acids of the invention are preferably *H.influenzae* nucleic acids.

Nucleic acids of the invention may be prepared in many ways *e.g.* by chemical synthesis (*e.g.* phosphoramidite synthesis of DNA) in whole or in part, by digesting longer nucleic acids using nucleases (*e.g.* restriction enzymes), by joining shorter nucleic acids or nucleotides (*e.g.* using ligases or polymerases), from genomic or cDNA libraries, *etc.*

Nucleic acid of the invention may be attached to a solid support (*e.g.* a bead, plate, filter, film, slide, microarray support, resin, *etc.*). Nucleic acid of the invention may be labelled *e.g.* with a radioactive or fluorescent label, or a biotin label. This is particularly useful where the nucleic acid is to be used in detection techniques *e.g.* where the nucleic acid is a primer or as a probe.

The term "nucleic acid" includes in general means a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. It includes DNA, RNA, DNA/RNA hybrids. It also includes DNA or RNA analogs, such as those containing modified backbones (*e.g.* peptide nucleic acids (PNAs) or phosphorothioates) or modified bases. Thus the invention includes mRNA, tRNA, rRNA, ribozymes, DNA, cDNA, recombinant nucleic acids, branched nucleic acids, plasmids, vectors, probes, primers, *etc.*. Where nucleic acid of the invention takes the form of RNA, it may or may not have a 5' cap.

Nucleic acids of the invention comprise meningococcal sequences as defined above, but they may also comprise non-meningococcal sequences (*e.g.* in nucleic acids of formula 5'-X-Y-Z-3', as defined above). This is particularly useful for primers, which may thus comprise a first sequence complementary to a PCAV nucleic acid target and a second sequence which is not complementary to the nucleic acid target. Any such non-complementary sequences in the primer are preferably 5' to the complementary sequences. Typical non-complementary sequences comprise restriction sites or promoter sequences.

Nucleic acids of the invention can be prepared in many ways *e.g.* by chemical synthesis (at least in part), by digesting longer nucleic acids using nucleases (*e.g.* restriction enzymes), by joining shorter nucleic acids (*e.g.* using ligases or polymerases), from genomic or cDNA libraries, *etc.*

Nucleic acids of the invention may be part of a vector *i.e.* part of a nucleic acid construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "cloning vectors" which are designed for isolation, propagation and replication of inserted nucleotides, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, "viral vectors" which is designed to result in the production of a recombinant virus or virus-like particle, or "shuttle vectors", which comprise the attributes of more than one type of vector. Preferred vectors are plasmids. A "host cell" includes an individual cell or cell culture which can be or has been a recipient of exogenous nucleic acid. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. Host cells include cells transfected or infected *in vivo* or *in vitro* with nucleic acid of the invention.

Where a nucleic acid is DNA, it will be appreciated that "U" in a RNA sequence will be replaced by "T" in the DNA. Similarly, where a nucleic acid is RNA, it will be appreciated that "T" in a DNA sequence will be replaced by "U" in the RNA.

The term "complement" or "complementary" when used in relation to nucleic acids refers to Watson-Crick base pairing. Thus the complement of C is G, the complement of G is C, the complement of A is T (or U), and the complement of T (or U) is A. It is also possible to use bases such as I (the purine inosine) *e.g.* to complement pyrimidines (C or T). The terms also imply a direction – the complement of 5'-ACAGT-3' is 5'-ACTGT-3' rather than 5'-TGTCA-3'.

Nucleic acids of the invention can be used, for example: to produce polypeptides; as hybridization probes for the detection of nucleic acid in biological samples; to generate additional copies of the nucleic acids; to generate ribozymes or antisense oligonucleotides; as single-stranded DNA primers or probes; or as triple-strand forming oligonucleotides.

The invention provides a process for producing nucleic acid of the invention, wherein the nucleic acid is synthesised in part or in whole using chemical means.

The invention provides vectors comprising nucleotide sequences of the invention (*e.g.* cloning or expression vectors) and host cells transformed with such vectors.

The invention also provides a kit comprising primers (*e.g.* PCR primers) for amplifying a template sequence contained within a meningococcus nucleic acid sequence, the kit comprising a first primer and a second primer, wherein the first primer is substantially complementary to said template sequence and the second primer is substantially complementary to a complement of said template sequence, wherein the parts of said primers which have substantial complementarity define the termini of the template sequence to be amplified. The first primer and/or the second primer may include a detectable label (*e.g.* a fluorescent label).

The invention also provides a kit comprising first and second single-stranded oligonucleotides which allow amplification of a meningococcal template nucleic acid sequence contained in a single- or double-stranded nucleic acid (or mixture thereof), wherein: (a) the first oligonucleotide comprises a primer sequence which is substantially complementary to said template nucleic acid sequence; (b) the second oligonucleotide comprises a primer sequence which is substantially complementary to the complement of said template nucleic acid sequence; (c) the first oligonucleotide and/or the second oligonucleotide comprise(s) sequence which is not compementary to said template nucleic acid; and (d) said primer sequences define the termini of the template sequence to be amplified. The non-complementary sequence(s) of feature (c) are preferably upstream of (*i.e.* 5' to) the primer sequences. One or both of these (c) sequences may comprise a restriction site [*e.g.* ref.33] or a promoter sequence [*e.g.* 34]. The first oligonucleotide and/or the second oligonucleotide may include a detectable label (*e.g.* a fluorescent label).

The template sequence may be any part of a genome sequence.

The invention provides a process for detecting nucleic acid of the invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridising conditions to form duplexes; and (b) detecting said duplexes.

The invention provides a process for detecting meningococcus in a biological sample (*e.g.* blood), comprising the step of contacting nucleic acid according to the invention with the biological sample under hybridising conditions. The process may involve nucleic acid amplification (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA, *etc.*) or hybridisation (*e.g.* microarrays, blots, hybridisation with a probe in solution *etc.*).

The invention provides a process for preparing a fragment of a target sequence, wherein the fragment is prepared by extension of a nucleic acid primer. The target sequence and/or the primer are nucleic acids of the invention. The primer extension reaction may involve nucleic acid amplification (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA, *etc.*).

Nucleic acid amplification according to the invention may be quantitative and/or real-time.

For certain embodiments of the invention, nucleic acids are preferably at least 7 nucleotides in length (*e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300 nucleotides or longer).

For certain embodiments of the invention, nucleic acids are preferably at most 500 nucleotides in length (*e.g.* 450, 400, 350, 300, 250, 200, 150, 140, 130, 120, 110, 100, 90, 80, 75, 70, 65, 60, 55, 50, 45, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15 nucleotides or shorter).

Primers and probes of the invention, and other nucleic acids used for hybridization, are preferably between 10 and 30 nucleotides in length (*e.g.* 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides).

Pharmaceutical compositions

The invention provides compositions comprising: (a) polypeptide, antibody, and/or nucleic acid of the invention; and (b) a pharmaceutically acceptable carrier. These compositions may be suitable as immunogenic compositions, for instance, or as diagnostic reagents, or as vaccines. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

A 'pharmaceutically acceptable carriers' includes any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.*

Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in ref. 141.

Compositions of the invention may include an antimicrobial, particularly if packaged in a multiple dose format.

Compositions of the invention may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Compositions of the invention will generally include a buffer. A phosphate buffer is typical.

Compositions of the invention may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

Polypeptides of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include a vaccine adjuvant. Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* [*e.g.* see chapters 8 & 9 of ref. 35], or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [36].

Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100μg Al^{3+} per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 35; see also ref. 37] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 35]

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 38. Saponin formulations may also comprise a sterol, such as cholesterol [39].

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexs (ISCOMs) [chapter 23 of ref. 35]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 39-41. Optionally, the ISCOMS may be devoid of additional detergent [42].

A review of the development of saponin based adjuvants can be found in refs. 43 & 44.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 45-50. Virosomes are discussed further in, for example, ref. 51

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in

ref. 52. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22μm membrane [52]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [53,54].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 55 & 56.

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 57, 58 and 59 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 60-65.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [66]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 67-69. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 66 & 70-72.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 73 and as parenteral adjuvants in ref. 74. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 75-82. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 83, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [84], *etc.*) [85], interferons (*e.g.* interferon-

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [86] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [87].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of

-toxic (*e.g.* a

-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 35)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 88-90.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [91]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [92] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [93]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in refs. 94 and 95.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (*e,g.* "Resiquimod 3M"), described further in refs. 96 and 97.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [98]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [99]; (3) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol; (4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [100]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [101]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 35.

The use of an aluminium hydroxide or aluminium phosphate adjuvant is particularly preferred, and antigens are generally adsorbed to these salts. Calcium phosphate is another preferred adjuvant.

The pH of compositions of the invention is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [102]. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses. Injectable compositions will usually be liquid solutions or suspensions. Alternatively, they may be presented in solid form (*e.g.* freeze-dried) for solution or suspension in liquid vehicles prior to injection.

Compositions of the invention may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage volumes can be routinely established, but a typical human dose of the composition for injection has a volume of 0.5ml.

Where a composition of the invention is to be prepared extemporaneously prior to use (*e.g.* where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and

physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials, and a typical quantity of each meningococcal saccharide antigen per dose is between 1μg and 10mg per antigen.

Pharmaceutical uses

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition of the invention. The patient may either be at risk from the disease themselves or may be a pregnant woman ('maternal immunisation').

The invention provides nucleic acid, polypeptide, or antibody of the invention for use as medicaments (*e.g.* as immunogenic compositions or as vaccines, including for use in treating or preventing disease and/or infection caused by meningococcus) or as diagnostic reagents. It also provides the use of nucleic acid, polypeptide, or antibody of the invention in the manufacture of: (i) a medicament for treating or preventing disease and/or infection caused by meningococcus; (ii) a diagnostic reagent for detecting the presence of meningococcus or of antibodies raised against meningococcus; and/or (iii) a reagent which can raise antibodies against meningococcus. Said meningococcus can be of any serogroup or strain, but is preferably in serogroup B. Said disease may be, for instance, bacterial meningitis (and particularly meningococcal meningitis) or septicaemia.

The patient is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or teenager *e.g.* ages 0-18 years; where the vaccine is for therapeutic use, the human is preferably an adult *e.g.* aged 18-55 years. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

One way of checking efficacy of therapeutic treatment involves monitoring meningococcal infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against an administered polypeptide after administration. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (*e.g.* children 12-16 months age, or animal models) and then determining standard parameters including ELISA titres (GMT) of IgG. These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before administration of the composition. Where more than one dose of the composition is administered, more than one post-administration determination may be made. A standard method for assessing prophylactic efficacy for meningococci is the serum bactericidal assay (SBA). Administration preferably results in an increase in SBA titre for the relevant serogroup of at least 4-fold, and preferably at least 8-fold, measured with human complement [103]. If rabbit complement is used to measure SBA titres then the titre increase is preferably at least 128-fold.

Administration of polypeptide antigens is a preferred method of treatment for inducing immunity. Administration of antibodies of the invention is another preferred method of treatment. This method of passive immunisation is particularly useful for newborn children or for pregnant women. This method will typically use monoclonal antibodies, which will be humanised or fully human.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral, vaginal, topical, transdermal, intranasal, ocular, aural, pulmonary or other mucosal administration. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

Bacterial infections affect various areas of the body and so compositions may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition be prepared for oral administration *e.g.* as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as spray, drops, gel or powder [*e.g.* refs 104 & 105].

Further antigenic components of compositions of the invention

The invention also provides a composition comprising a polypeptide or the invention and one or more of the following further antigens:

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y (preferably all four), such as the oligosaccharide disclosed in ref. 106 from serogroup C [see also ref. 107] or the oligosaccharides of ref. 108.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 109, 110, 111].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 112, 113].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 113, 114].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 115] *e.g.* the CRM_{197} mutant [*e.g.* 116].

- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 115].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 117 & 118].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 107].
- polio antigen(s) [*e.g.* 119, 120] such as IPV.
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 115].
- influenza antigen(s) [*e.g.* chapter 19 of ref. 115], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [*e.g.* 121].
- an protein antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 122, 123].
- a saccharide antigen from *Streptococcus agalactiae* (group B streptococcus).
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 123, 124, 125].
- an antigen from *Staphylococcus aureus* [*e.g.* 126].

The composition may comprise one or more of these further antigens.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [118]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. DTP combinations are thus preferred.

Saccharide antigens are preferably in the form of conjugates. Carrier proteins for the conjugates include diphtheria toxin, tetanus toxin, the *N.meningitidis* outer membrane protein [127], synthetic peptides [128,129], heat shock proteins [130,131], pertussis proteins [132,133], protein D from *H.influenzae* [134], cytokines [135], lymphokines [135], streptococcal proteins, hormones [135], growth factors [135], toxin A or B from *C.difficile* [136], iron-uptake proteins [137], *etc.* A preferred carrier protein is the CRM197 diphtheria toxoid [138].

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using proteins antigens in the immunogenic compositions of the invention, nucleic acid (preferably DNA *e.g.* in the form of a plasmid) encoding the antigen may be used.

Antigens are preferably adsorbed to an aluminium salt.

Screening methods

The invention provides a process for determining whether a test compound binds to a polypeptide of the invention. If a test compound binds to a polypeptide of the invention and this binding inhibits the

life cycle of meningococcus, then the test compound can be used as an antibiotic or as a lead compound for the design of antibiotics. The process will typically comprise the steps of contacting a test compound with a polypeptide of the invention, and determining whether the test compound binds to said polypeptide. Preferred polypeptides of the invention for use in these processes are enzymes (*e.g.* tRNA synthetases), membrane transporters and ribosomal polypeptides. Suitable test compounds include polypeptides, polypeptides, carbohydrates, lipids, nucleic acids (*e.g.* DNA, RNA, and modified forms thereof), as well as small organic compounds (*e.g.* MW between 200 and 2000 Da). The test compounds may be provided individually, but will typically be part of a library (*e.g.* a combinatorial library). Methods for detecting a binding interaction include NMR, filter-binding assays, gel-retardation assays, displacement assays, surface plasmon resonance, reverse two-hybrid *etc.* A compound which binds to a polypeptide of the invention can be tested for antibiotic activity by contacting the compound with meningococcus bacteria and then monitoring for inhibition of growth. The invention also provides a compound identified using these methods.

Preferably, the process comprises the steps of: (a) contacting a polypeptide of the invention with one or more candidate compounds to give a mixture; (b) incubating the mixture to allow polypeptide and the candidate compound(s) to interact; and (c) assessing whether the candidate compound binds to the polypeptide or modulates its activity.

Once a candidate compound has been identified *in vitro* as a compound that binds to a polypeptide of the invention then it may be desirable to perform further experiments to confirm the *in vivo* function of the compound in inhibiting bacterial growth and/or survival. Thus the method comprise the further step of contacting the compound with a meningococcus and assessing its effect.

The polypeptide used in the screening process may be free in solution, affixed to a solid support, located on a cell surface or located intracellularly. Preferably, the binding of a candidate compound to the polypeptide is detected by means of a label directly or indirectly associated with the candidate compound. The label may be a fluorophore, radioisotope, or other detectable label.

General

The invention provides a computer-readable medium (*e.g.* a floppy disk, a hard disk, a CD-ROM, a DVD *etc.*) and/or a computer memory and/or a computer database containing one or more of the sequences in the sequence listing.

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Identity between polypeptides is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*. Identity between sequences is preferably also determined by the Smith-Waterman homology search algorithm.

The N-terminus residues in the amino acid sequences in the sequence listing are given as the amino acid encoded by the first codon in the corresponding nucleotide sequence. Where the first codon is not ATG, it will be understood that it will be translated as methionine when the codon is a start codon, but will be translated as the indicated non-Met amino acid when the sequence is at the C-terminus of a fusion partner. The invention specifically discloses and encompasses each of the amino acid sequences of the sequence listing having a N-terminus methionine residue (*e.g.* a formyl-methionine residue) in place of any indicated non-Met residue.

Alternative start codons can be used in biology. The amino acid sequences in the sequence listing are based on particular start codons, but downstream start codons may alternatively be used. Thus the invention specifically discloses and encompasses each of the amino acid sequences of the sequence listing, starting at any methionine residue from the sequence that is downstream of the N-terminal residue shown in the sequence listing (*e.g.* SEQ ID NOs: 5 & 10).

As indicated in the above text, nucleic acids and polypeptides of the invention may include sequences that:

(a) are identical (*i.e.* 100% identical) to the sequences disclosed in the sequence listing;

(b) share sequence identity with the sequences disclosed in the sequence listing;

(c) have 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 single nucleotide or amino acid alterations (deletions, insertions, substitutions), which may be at separate locations or may be contiguous, as compared to the sequences of (a) or (b); and

(d) when aligned with a particular sequence from the sequence listing using a pairwise alignment algorithm, a moving window of x monomers (amino acids or nucleotides) moving from start (N-terminus or 5') to end (C-terminus of 3'), such that for an alignment that extends to p monomers (where $p>x$) there are $p-x+1$ such windows, each window has at least $x \cdot y$ identical aligned monomers, where: x is selected from 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200; y is selected from 0.50, 0.60, 0.70, 0.75, 0.80, 0.85, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99; and if $x \cdot y$ is is not an integer then it is rounded up to the nearest integer. The preferred pairwise alignment algorithm is the Needleman-Wunsch global alignment algorithm [139], using default parameters (*e.g.* with Gap opening penalty = 10.0, and with Gap extension penalty = 0.5, using the EBLOSUM62 scoring matrix). This algorithm is conveniently implemented in the *needle* tool in the EMBOSS package [140].

The nucleic acids and polypeptides of the invention may additionally have further sequences to the N-terminus/5' and/or C-terminus/3' of these sequences (a) to (d).

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references 141-148, *etc.*

MODES FOR CARRYING OUT THE INVENTION

Various encoded amino acid sequences have been identified in the genome of the M04-240196 strain of serogroup B *N.meningitidis*. 39 of them have been selected as useful antigens, based on various criteria, and their gene and amino acid sequences are given in the sequence listing.

Predicted biological functions are given in Table I, but the antigens' precise roles in meningococcus biology are not as important as their ability to function as immunogens. Table I also notes the closest match in the published serogroup A and B genomes in references 6 and 8, as well as in the unpublished genome of serogroup C strain FAM18. Where a sequence has more than 95% identity to a known sequence (and particularly where it has 100% identity) then the invention is more concerned with identifying the protein's useful antigenic properties than identifying the protein *per se*.

In addition to the annotations and comparisons, further features of interest include: B269_17 contains an intein domain; B269_34 has a junction sequence at its C-terminus; a transmembrane domain is present in B269_05, B269_10, B269_18, B269_24; and five transmembrane domains are present in B269_15 and B269_29.

Using the sequence information herein, the proteins can readily be expressed in recombinant hosts and used to generate immune responses using techniques known in the art.

For instance, sequences encoding B269_ proteins 11, 13, 14, 15, 17, 24, 25, 26, 29, 31, 32, 34, 36, 37, 51, 52, 53, 54, 55, 56, 57, 58 and 59 were inserted into expression vectors with a C-terminal poly-histidine tag. B269_ proteins 14, 29, 31, 34, 37 were also expressed in a domain-truncated form. GST fusions of 37, 54, 55 and 57 were also prepared. Expressed proteins were purified from *E.coli*. Without any optimisation of expression, various degrees of purity were seen *e.g.* from 20% purity with domain B269_14 and B269_32, up to 95% purity with B269_51. Soluble expression was seen with B269_ proteins 13, 24, 25, 31_{domain}, 32, 51, 53 and 56.

Antibodies were raised against expressed proteins by injecting them into mice with Freund's complete adjuvant or aluminium hydroxide. Antisera were then used in western blots or FACS binding assays against meningococci. The following B269_ proteins could be detected by western blot: 13; 25; 29_{domain}; 31; 34_{domain}; 51; 52; and 53. In addition, the following proteins could be detected in the blots at particular MWs: 11 (40kDa); 24 (20kDa); and 26 (28kDa). FACS revealed the following proteins: 17; 24; 25; 26; 29_{domain}; 34_{domain}; and 53.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

TABLE I — Annotations

aa = length of polypeptide

B269	SEQ ID	aa	Localisation *	Annotation
01	2	588	O	PilC protein
11	4	320	O	putative MafA-like adhesin
13	6	387	C	Cupin superfamily protein
14	8	420	I	membrane-fusion protein
15	10	670	I	peptidase, C39 family
16	12	331	P	putative peptidyl-prolyl cis-trans isomerase
17	14	209	S	conserved hypothetical protein
18	16	265	O	opacity protein
19	18	680	I	transferrin-binding protein 2
20	20	1370	S	hemagglutinin-hemolysin-related protein
21	22	734	S	hemagglutinin-hemolysin-related protein
22	24	887	S	hemagglutinin-hemolysin-related protein
23	26	794	S	hemagglutinin-hemolysin-related protein
24	28	206	C	conserved hypothetical protein
25	30	1502	S	HlyJ haemolysin-like protein
26	32	257	C	conserved hypothetical protein TIGR00294
27	34	237	I	putative thiosulphate sulphur transferase
28	36	402	C	probable: Putative GlcNAc transferase19
29	38	297	O	conserved hypothetical protein
30	40	226	C	opacity protein Opa115
31	42	588	O	YadA-like C-terminal region family
32	44	201	C	conserved hypothetical protein
33	46	337	I	putative glycosyltransferase
34	48	529	C	Iron-regulated protein frpA
35	50	676	C	transferrin/lactoferrin binding protein B
36	52	203	C	mucin
37	54	340	C	conserved hypothetical protein
38	56	376	I	putative two component sensor kinase196
39	58	346	P	ATP-binding region, ATPase-like:Histidine kinase A, N-terminal
41	60	1026	P	PilC protein
42	62	333	O	conserved hypothetical protein
43	64	229	C	glycosyl transferase, group 2 family protein
44	66	208	C	conserved hypothetical protein
45	68	476	C	mafB protein
46	70	229	C	adhesin MafB
47	72	432	O	transferrin binding protein B subunit19
48	74	809	S	hemolysin-hemagglutinin-like protein HecA precursor, putative
49	76	783	S	Possible hemagglutinin (DUF637) family1
50	78	300	S	hemagglutinin-hemolysin-related protein

* Localisation key: O = outer membrane; C = cytoplasm; I = inner membrane; P = periplasmic space; S = secreted

TABLE II — Relationship to other meningococcal sequences [6,8]

'MC58' = closest match from reference 6.

Columns (a) to (c) are % matches to other sequences: (B) = ref 6; (A) = ref 8; (c) = strain FAM18.

B269	SEQ ID	MC58	(B)	(A)	(C)
01	2	NMB0049	77.6	78.2	73.1
11	4	NMB0652	62,7	100	98,4
13	6	NMB1786	51	48.4	80.3
14	8	NMB0097	51.1	59.9	100
15	10	NMB0098	64	28.9	94.9
16	12	NMB0281	85.4	92.1	93.5
17	14	NMB0369	83.2	61.7	91.9
18	16	NMB0442	86.4	81.5	87.1
19	18	NMB0460	71.5	70.9	41.8
20	20	NMB1779	67.4	70	97.4
21	22	NMB1775	82.6	87.9	96
22	24	NMB1779	81.9	80.5	95.2
23	26	NMB1775	79.2	89.2	88.9
24	28	NMB0515	66.1	99.5	93.7
25	30	NMB0585	85,5	91	90,9
26	32	NMB0803	86.8	86.8	86.8
27	34	NMB0841	84	95.4	88.1
28	36	NMB0846	89	56.1	85.1
29	38	NMB0888	87.9	79.8	85.2
30	40	NMB1636	80.4	81.6	92
31	42	NMB0992	87.4	90.1	94.6
32	44	none	41.2	41.2	70.9
33	46	NMB1255	73	95.4	97.5
34	48	NMB1415	89.5	80.5	98.7
35	50	NMB1541	68.3	78.2	56.4
36	52	NMB0891	49	53.4	78.6
37	54	none	30.7	33.3	100
38	56	NMB1606	82.2	80.1	87
39	58	NMB1606	83	85.9	90.9
41	60	NMB1847	80.4	81.6	78.2
42	62	NMB1870	87.4	87.4	74.2
43	64	NMB1929	48.2	48.2	48.2
44	66	NMB1992	89.9	81.5	92.8
45	68	NMB2105	89.7	88.5	95.9
46	70	NMB2105	88	96.7	89.9
47	72	NMB2132	76,5	68,6	68,7
48	74	NMB0493	85,4	55	68,2
49	76	NMB1775	67,2	82,9	99,4
50	78	NMB1779	51,2	84	83,3

REFERENCES (the contents of which are hereby incorporated by reference)

[1] WO99/24578.
[2] WO99/36544.
[3] WO99/57280.
[4] WO00/22430.
[5] WO00/66791.
[6] Tettelin *et al.* (2000) *Science* 287:1809-16.
[7] Pizza *et al.* (2000) *Science* 287:1-1816-20.
[8] Parkhill *et al.* (2000) *Nature* 404:502-8.
[9] Geysen *et al.* (1984) *PNAS USA* 81:3998-4002.
[10] Carter (1994) *Methods Mol Biol* 36:207-23.
[11] Jameson, BA *et al.* 1988, *CABIOS* 4(1):181-186.
[12] Raddrizzani & Hammer (2000) *Brief Bioinform* 1(2):179-89.
[13] De Lalla *et al.* (1999) *J. Immunol.* 163:1725-29.
[14] Brusic *et al.* (1998) *Bioinformatics* 14(2):121-30
[15] Meister *et al.* (1995) *Vaccine* 13(6):581-91.
[16] Roberts *et al.* (1996) *AIDS Res Hum Retroviruses* 12(7):593-610.
[17] Maksyutov & Zagrebelnaya (1993) *Comput Appl Biosci* 9(3):291-7.
[18] Feller & de la Cruz (1991) *Nature* 349(6311):720-1.
[19] Hopp (1993) *Peptide Research* 6:183-190.
[20] Welling *et al.* (1985) *FEBS Lett.* 188:215-218.
[21] Davenport *et al.* (1995) *Immunogenetics* 42:392-297.
[22] Bodanszky (1993) *Principles of Peptide Synthesis* (ISBN: 0387564314).
[23] Fields *et al.* (1997) *Meth Enzymol* 289: *Solid-Phase Peptide Synthesis.* ISBN: 0121821900.
[24] Chan & White (2000) *Fmoc Solid Phase Peptide Synthesis.* ISBN: 0199637245.
[25] Kullmann (1987) *Enzymatic Peptide Synthesis.* ISBN: 0849368413.
[26] Ibba (1996) *Biotechnol Genet Eng Rev* 13:197-216.
[27] Breedveld (2000) *Lancet* 355(9205):735-740.
[28] Gorman & Clark (1990) *Semin. Immunol.* 2:457-466.
[29] Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual.*
[30] *Short protocols in molecular biology* (4th ed, 1999) Ausubel *et al.* eds. ISBN 0-471-32938-X.
[31] US patent 5,707,829
[32] *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.* eds., 1987) Supplement 30.
[33] EP-B-0509612.
[34] EP-B-0505012.
[35] *Vaccine Design...* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.
[36] WO00/23105.
[37] WO90/14837.
[38] US patent 5,057,540.
[39] WO96/33739.
[40] EP-A-0109942.

[41] WO96/11711.
[42] WO00/07621.
[43] Barr *et al.* (1998) *Advanced Drug Delivery Reviews* 32:247-271.
[44] Sjolanderet *et al.* (1998) *Advanced Drug Delivery Reviews* 32:321-338.
[45] Niikura *et al.* (2002) *Virology* 293:273-280.
[46] Lenz *et al.* (2001) *J Immunol* 166:5346-5355.
[47] Pinto *et al.* (2003) *J Infect Dis* 188:327-338.
[48] Gerber *et al.* (2001) *Virol* 75:4752-4760.
[49] WO03/024480
[50] WO03/024481
[51] Gluck *et al.* (2002) *Vaccine* 20:B10-B16.
[52] EP-A-0689454.
[53] Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.
[54] Evans *et al.* (2003) *Expert Rev Vaccines* 2:219-229.
[55] Meraldi *et al.* (2003) *Vaccine* 21:2485-2491.
[56] Pajak *et al.* (2003) *Vaccine* 21:836-842.
[57] Kandimalla *et al.* (2003) *Nucleic Acids Research* 31:2393-2400.
[58] WO02/26757.
[59] WO99/62923.
[60] Krieg (2003) *Nature Medicine* 9:831-835.
[61] McCluskie *et al.* (2002) *FEMS Immunology and Medical Microbiology* 32:179-185.
[62] WO98/40100.
[63] US patent 6,207,646.
[64] US patent 6,239,116.
[65] US patent 6,429,199.
[66] Kandimalla *et al.* (2003) *Biochemical Society Transactions* 31 (part 3):654-658.
[67] Blackwell *et al.* (2003) *J Immunol* 170:4061-4068.
[68] Krieg (2002) *Trends Immunol* 23:64-65.
[69] WO01/95935.
[70] Kandimalla *et al.* (2003) *BBRC* 306:948-953.
[71] Bhagat *et al.* (2003) *BBRC* 300:853-861.
[72] WO03/035836.
[73] WO95/17211.
[74] WO98/42375.
[75] Beignon *et al.* (2002) *Infect Immun* 70:3012-3019.
[76] Pizza *et al.* (2001) *Vaccine* 19:2534-2541.
[77] Pizza *et al.* (2000) *Int J Med Microbiol* 290:455-461.
[78] Scharton-Kersten *et al.* (2000) *Infect Immun* 68:5306-5313.
[79] Ryan *et al.* (1999) *Infect Immun* 67:6270-6280.
[80] Partidos *et al.* (1999) *Immunol Lett* 67:209-216.
[81] Peppoloni *et al.* (2003) *Expert Rev Vaccines* 2:285-293.

[82] Pine *et al.* (2002) *J Control Release* 85:263-270.
[83] Domenighini *et al.* (1995) *Mol Microbiol* 15:1165-1167.
[84] WO99/40936.
[85] WO99/44636.
[86] Singh *et al]* (2001) *J Cont Release* 70:267-276.
[87] WO99/27960.
[88] US patent 6,090,406
[89] US patent 5,916,588
[90] EP-A-0626169.
[91] WO99/52549.
[92] WO01/21207.
[93] WO01/21152.
[94] Andrianov *et al.* (1998) *Biomaterials* 19:109-115.
[95] Payne *et al.* (1998) *Adv Drug Delivery Review* 31:185-196.
[96] Stanley (2002) *Clin Exp Dermatol* 27:571-577.
[97] Jones (2003) *Curr Opin Investig Drugs* 4:214-218.
[98] WO99/11241.
[99] WO94/00153.
[100] WO98/57659.
[101] European patent applications 0835318, 0735898 and 0761231.
[102] WO03/009869.
[103] *W.H.O. Tech. Rep. Ser.* 594:51, 1976.
[104] Almeida & Alpar (1996) *J. Drug Targeting* 3:455-467.
[105] Agarwal & Mishra (1999) *Indian J Exp Biol* 37:6-16.
[106] Costantino *et al.* (1992) *Vaccine* 10:691-698.
[107] Costantino *et al.* (1999) *Vaccine* 17:1251-1263.
[108] WO03/007985.
[109] Watson (2000) *Pediatr Infect Dis J* 19:331-332.
[110] Rubin (2000) *Pediatr Clin North Am* 47:269-285, v.
[111] Jedrzejas (2001) *Microbiol Mol Biol Rev* 65:187-207.
[112] Bell (2000) *Pediatr Infect Dis J* 19:1187-1188.
[113] Iwarson (1995) *APMIS* 103:321-326.
[114] Gerlich *et al.* (1990) *Vaccine* 8 Suppl:S63-68 & 79-80.
[115] *Vaccines* (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0.
[116] Del Guidice *et al.* (1998) *Molecular Aspects of Medicine* 19:1-70.
[117] Gustafsson *et al.* (1996) *N. Engl. J. Med.* 334:349-355.
[118] Rappuoli *et al.* (1991) *TIBTECH* 9:232-238.
[119] Sutter *et al.* (2000) *Pediatr Clin North Am* 47:287-308.
[120] Zimmerman & Spann (1999) *Am Fam Physician* 59:113-118, 125-126.
[121] McMichael (2000) *Vaccine* 19 Suppl 1:S101-107.
[122] Schuchat (1999) *Lancet* 353(9146):51-6.

[123] WO02/34771.

[124] Dale (1999) *Infect Dis Clin North Am* 13:227-43, viii.

[125] Ferretti *et al.* (2001) *PNAS USA* 98: 4658-4663.

[126] Kuroda *et al.* (2001) *Lancet* 357(9264):1225-1240; see also pages 1218-1219.

[127] EP-A-0372501

[128] EP-A-0378881

[129] EP-A-0427347

[130] WO93/17712

[131] WO94/03208

[132] WO98/58668

[133] EP-A-0471177

[134] WO00/56360

[135] WO91/01146

[136] WO00/61761

[137] WO01/72337

[138] *Research Disclosure*, 453077 (Jan 2002)

[139] Needleman& Wunsch (1970) *J. Mol. Biol.* 48, 443-453.

[140] Rice *et al.* (2000) *Trends Genet* 16:276-277.

[141] Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th edition, ISBN: 0683306472.

[142] *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.)

[143] *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications)

[144] Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989).

[145] *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997)

[146] *Short Protocols in Molecular Biology*, 4th ed. (Ausubel *et al.* eds., 1999, John Wiley & Sons)

[147] *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream *et al.*, eds., 1998, Academic Press)

[148] *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag)

CLAIMS

1. A polypeptide comprising an amino acid sequence that has at least 75% sequence identity to one or more of SEQ ID NOS: 32, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78.
2. The polypeptide of claim 1, comprising one or more of amino acid sequences SEQ ID NOS: 32, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78.
3. A polypeptide comprising a fragment of at least 7 consecutive amino acids from one or more of SEQ ID NOS: 32, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78..
4. The polypeptide of claim 3, wherein the fragment comprises a T-cell or a B-cell epitope from the SEQ ID NO: amino acid sequence.
5. Antibody that binds to the polypeptide of any preceding claim.
6. Antibody of claim 5, wherein the antibody is a monoclonal antibody.
7. Nucleic acid comprising an nucleotide sequence that has at least 75% sequence identity to one or more of SEQ ID NOS: 31, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77.
8. Nucleic acid of claim 7, comprising an nucleotide sequence selected from SEQ ID NOS: 31, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77.
9. Nucleic acid that can hybridize to the nucleic acid of claim 8 under high stringency conditions.
10. Nucleic acid comprising a fragments of 10 or more consecutive nucleotides from one or more of SEQ ID NOS: 31, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77.
11. Nucleic acid encoding the polypeptide of any one of claims 1 to 4.
12. A composition comprising: (a) polypeptide, antibody, and/or nucleic acid of any preceding claim; and (b) a pharmaceutically acceptable carrier.
13. The composition of claim 12, further comprising a vaccine adjuvant.
14. Nucleic acid, polypeptide, or antibody of any one of claims 1 to 11 for use as a medicament.
15. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of the composition of claim 12.
16. The use of nucleic acid, polypeptide, or antibody of any one of claims 1 to 11 in the manufacture of a medicament for treating or preventing disease and/or infection caused by *Neisseria meningitidis*
17. The method of claim 15, or the use of claim 16, for preventing meningococcal meningitis.